Histochem Cell Biol (1997) 108:299–305

ORIGINAL PAPER

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Spectral karyotyping, a 24-colour FISH technique for the identification of chromosomal rearrangements

Accepted: 3 July 1997

Abstract Spectral karyotyping (SKY) is a new fluorescence in situ hybridisation (FISH) technique that refers to the molecular cytogenetic analysis of metaphase preparations by means of spectral microscopy. For SKY of human metaphase chromosomes, 24 chromosome-specific painting probes are used in just one FISH experiment. The probes are labelled by degenerate oligonucleotide-primed PCR using three fluorochromes and two haptens. Each probe is differentially labelled with one, two, three or four fluorescent dyes, resulting in a unique spectral signature for every chromosome. After in situ hybridisation and immunodetection, a spectral image is acquired using a conventional fluorescence light microscope equipped with a custom-designed triple-bandpass filter and the SpectraCube, which is able to retrieve spectral information for every pixel in a digital CCD image. The 24-colour display and chromosome classification are based on the unique emission spectra of the chromosomes. Together with chromosome banding information from an inverted DAPI or a G-banded metaphase, a comprehensive overview of chromosomal aberrations is presented.

Introduction

Screening cell karyotypes for chromosomal abnormalities is an integral part of the diagnosis of human cancers and congenital diseases. Recurrent chromosomal aberrations are often the result of events at the molecular level and the affected chromosomal regions potentially contain genes involved in the disease. Routine karyotype analysis to date is based on cytochemical chromosome banding techniques which provide hallmarks for clinical diagnosis. Although many important karyotypic changes have been found by means of chromosome banding, numerous technical factors may hamper full characterisation of complex genomes. The mitotic index must be high and the chromosomes must be of good quality to allow successful banding analysis. In many instances, and for solid tumours in particular, this may be a hurdle too high to overcome. Even if a metaphase preparation is optimal, the identification of complex chromosomal rearrangements could be extremely difficult or impossible. Also, chromosomal structures that do not display bands at all, such as homogeneously staining regions (hsr's) and double-minute chromosomes, remain unidentified.

With the advent of fluorescence in situ hybridisation (FISH) the methodological gap between molecular genetics and conventional banding analysis has been closed. FISH enables one to confirm suspected chromosomal aberrations based on prior banding studies. Over the past decade, cytogeneticists have 'gone fishing' for almost every kind of chromosomal aberration (LeBeau 1993): numerical chromosomal aberrations have been detected in interphase cells (e.g. Ried et al. 1992a) and tissue sections using centromere-specific probes (e.g. Hopman et al. 1988); deletions, amplifications, insertions, inversions and translocations have been visualised using locus-specific probes on metaphase cells (e.g. Ried et al. 1992b) or fibre-FISH preparations (Florijn et al. 1995); and marker chromosomes unrecognised by conventional banding have been identified using whole chromosome painting probes (Carter 1994). Although FISH has developed into a widespread and highly valued tool in cytogenetic diagnosis and research, a major disadvantage is that, for the selection of FISH probes, a presumption is required of what the aberration might be. This approach is prone to mistakes and may lead to time-consuming series of experiments. It is clear that molecular cytogeneticists would benefit from a FISH-based technique that screens the whole genome for chromosomal aberrations in just one experiment.

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The colour of fluorescent light is determined by the wavelength of emitted photons. For biological applications of fluorescence microscopy, fluorochromes with different emission wavelengths, i.e. different colours, have been developed. Various strategies have been employed to develop FISH detection of more than one target sequence simultaneously in the same sample. In 1990, Nederlof et al. published the first combinatorial multi-colour FISH method using three differentially labelled centromere probes which were indirectly detected by immunocytochemical methods to generate four FISH signals simultaneously in the same cell. Image registration was achieved by taking consecutive photomicrographs through fluorochrome-specific optical filters. With the development of direct FISH techniques and the expansion of available haptens, Ried et al. (1992c) and Wiegant et al. (1993) elaborated the combinatorial labelling approach to the point that seven DNA targets could be detected in the same cell with three fluorochromes. Simultaneous visualisation required digital image registration of three grey-scale images taken consecutively through three single-bandpass optical filters. Seven pseudo-colours were then assigned, based on the combinatorial labelling scheme. The number of colours was further increased to 12 by combining haptens and fluorochromes in different ratios and the use of multi-bandpass filters (Dauwerse et al. 1992). At that time, molecular cytogeneticists were very optimistic about completing the painting of human chromosomes in 24 different colours (Ledbetter 1992). In theory, increasing the number of fluorochromes to five in an equimolar combinatorial labelling scheme would create 31 different colours. It was not until last year, however, that two papers on 24-colour FISH were published. The first, called multiplex-FISH or M-FISH (Speicher et al. 1996), pursued the existing strategy for image registration and analysis: five images are sequentially acquired through fluorochrome-specific optical filters, followed by assigning pseudo-colours based on grey-scale intensity values using custom-designed software. The second technique, called spectral karyotyping (SKY; Schröck et al. 1996) uses a new optical approach based on a single exposure through a triple-bandpass optical filter and spectroscopic analysis. Spectral imaging is not dependent on fluorescence intensity but solely on spectral signatures created by combinatorial labelling. This paper will not only show the potential of SKY to elucidate chromosomal aberrations but will also focus on the cytochemical aspects of spectral karyotyping and will provide the current protocol for 24-colour combinatorial probe labelling, metaphase slide preparation, SKY in situ hybridisation and performing G-banding and SKY on the same specimen. In addition, it will touch upon the principles and practice of spectral imaging and image processing.

Methodology

Metaphase preparations

Metaphase slides were prepared according to standard procedures. We were able to hybridise successfully cell metaphases from many cytogenetic laboratories in the United States of America and Europe, including different cell types such as bone marrow cells from haematological malignancies (Veldman et al. 1997), blood lymphocytes from clinical cases (E. Schröck, manuscript submitted for publication), primary solid tumour cultures (H. Padilla-Nash, personal communication), and established solid tumour cell lines (Garini et al. 1996; Schröck et al. 1996). For good quality tumour metaphase preparations satisfactory results have been obtained applying recommendations reported by Zimonjic and Popescu (1994). Metaphase slides can be stored at –20°C in 100% ethanol and will allow successful SKY hybridisation for up to 1 month. After 1 month the slides should be stored with desiccant at –80°C, which will produce good results for up to several months. For comprehensive analysis of aberrant chromosomes, some quality control parameters for cell metaphases are applied. On the one hand, overlapping chromosomes should be avoided because mixed spectra in overlapping regions cannot be reliably classified. On the other hand metaphases may overspread and some chromosomes may even wash off, so that the full metaphase cannot be analysed. Furthermore, long, extended chromosomes are preferred because that will increase the spatial resolution of chromosome analysis.

Degenerate oligonucleotide-primed PCR labelling and probe preparation

Chromosome-specific painting probes are obtained by flow sorting and amplified by two rounds of degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR; Telenius et al. 1992). To assure hybridisation specificity, every new batch of flow-sorted chromosomes is tested in 24 single-paint FISH experiments. The secondary amplification product is used for DOP-PCR labelling for SKY. As a rule, the secondary product is used for labelling even though SKY has been successfully performed using quaternary-labelled PCR product. The current labelling scheme is shown in Table 1. Based on fluorescence peak emission wavelength, quantum yield and fading properties we have chosen the fluorochromes SpectrumGreen, SpectrumOrange (dUTP conjugates; Vysis, Downers Grove, Ill.), Texas red (12-dUTP conjugate; Molecular Probes, Eugene, Ore.), Cy5 and Cy5.5 as the five components of the spectral signature. Cy5 and Cy5.5 labelling is performed indirectly through, respectively, biotin-16-dUTP and digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, Ind.). For biotin detection avidin-Cy5 (Amersham Life Sciences, Arlington Heights, Ill.) is used and for digoxigenin detection mouse anti-digoxin (Sigma, St. Louis, Mo.) followed by goat anti-mouse custom-conjugated to Cy5.5 (Amersham).

A unique spectral signature is created by differentially labelling each chromosome, adding up to a total of 57 individual PCR labelling reactions (Fig. 1). A single chro-

Table 1 Chromosome labelling scheme for spectral karyotyping. A total of 57 individual degenerate oligonucleotide-primed (*DOP*)-PCR labelling reactions are performed to create 24 unique emission spectra

Fluorochrome	Chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
SpectrumGreen	x		x		x		x	x	x			x	x					x		x	x		x	x
SpectrumOrange					x				x		x		x		x	x		x	x	x		x		x
Texas red	x			x	x	x						x		x	x			x				x	x	
Biotin/Cy5	x		x	x			x			x		x			x		x		x	x		x		x
Digoxigenin/Cy5.5		x	x		x	x			x	x		x				x					x	x		x
Number of DOP-PCRs	3	1	3	2	4	2	2	1	3	2	1	4	2	1	3	2	1	3	2	3	2	4	2	4

mosome painting probe (200–400 ng secondary PCR product) is labelled in a 100 µl total volume of PCR-II buffer (500 mM KCl, 100 mM TRIS-HCl, pH 8.3; Perkin-Elmer, Foster City, Calif.), 2 mM $MgCl_2$ (Perkin-Elmer), 200 µM dATP, 200 µM dGTP, 200 µM dCTP, 150 µM dTTP (all Boehringer Mannheim), 50 µM hapten- or fluorochrome-conjugated dUTP, 2 µM primer 6MW (5′-CCG ACT CGA GNN NNN NAT GTG G-3′ where *N* is any base; Midland Certified Reagent Company, Midland, Tex.), and 0.1 U Native *Taq* DNA polymerase (Perkin-Elmer). DOP-PCR labelling is performed in a thermocycler with heated lid (PTC-100/HB60; MJ Research, Watertown, Mass.) in 25 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 4 min. Final synthesis at 72°C was extended for 20 min. Fragment length (300–800 bp) and yield of the PCR products are checked by gel electrophoresis, typically by loading 1 µl labelled product on a 1% agarose gel (Fig. 1).

All 57 individual labelled chromosome painting probes are then pooled into one 6-ml reaction tube, mixed and subsequently aliquotted into 20 1.5-ml Eppendorf reaction tubes. Every Eppendorf now contains all 24 chromosome painting probes in the quantity needed for one SKY hybridisation. Each probe aliquot is precipitated with 50 µg human Cot1 DNA (Gibco/BRL Life Technologies, Grand Island, N.Y.) and 10 µg salmon testes DNA (Sigma). DNA pellets are dissolved in 15 µl hybridisation buffer containing 50% deionised formamide, 2×SSC (1×SSC is 150 mM NaCl, 15 mM sodium citrate), and 10% dextran sulphate. Probes can be stored up to 1 year at –20°C in the dark.

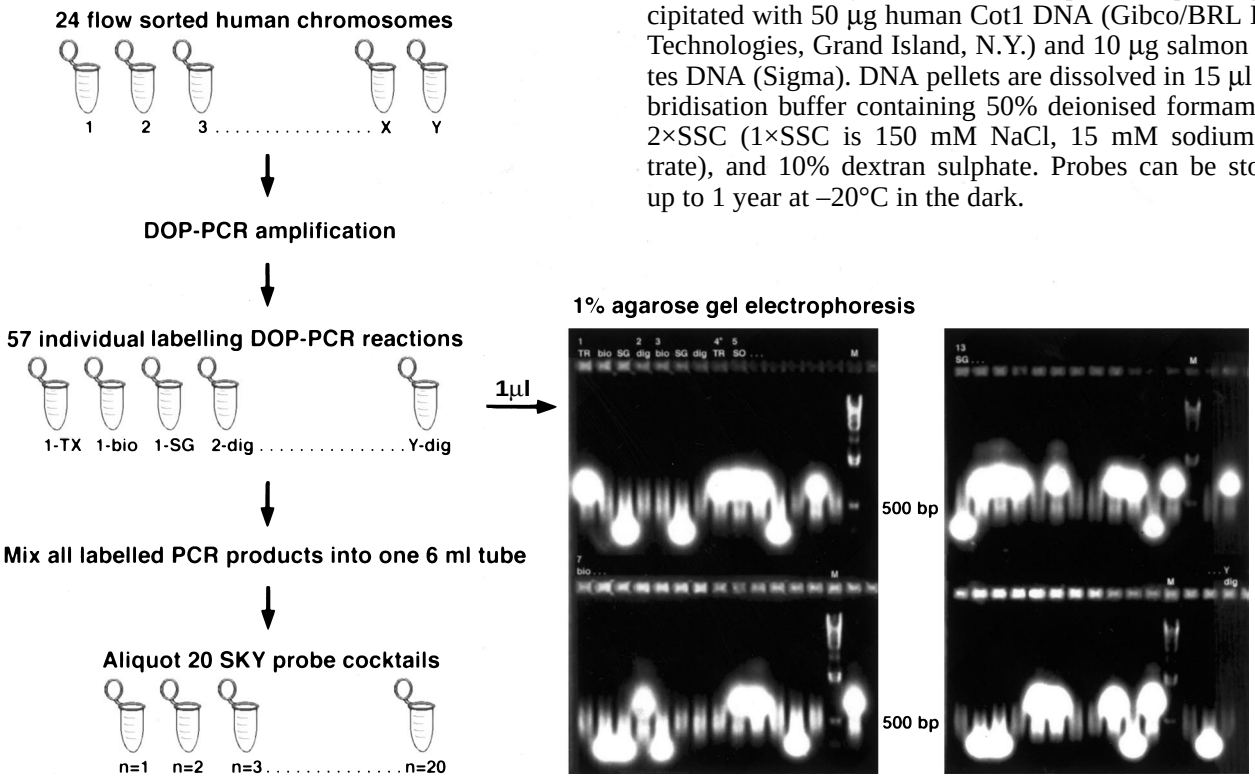

Fig. 1 Scheme of SKY probe preparation. The 1% agarose electrophoresis gel shows degenerate oligonucleotide-primed (*DOP*)-PCR-labelled whole chromosome painting probes as seen with UV illumination. Each lane represents one labelling with one fluorochrome or one hapten according Table 1. The numbers above the lanes refer to the specific chromosomes and the abbreviations refer to the fluorochromes used for singly labelling those chromosomes. For example, chromosome 1 is labelled with Texas red (*TR*), biotin (*bio*) and SpectrumGreen (*SG*); chromosome 2 with digoxigenin (*dig*). (*SO* SpectrumOrange) DNA fragments are between 300 and 800 bp in length as determined by the λ/*Hin*dIII DNA marker (*M*). Fluorescent dyes are lighting up very brightly at the DNA smear. Chromosome 4 labelling with digoxigenin is missing from this gel (*asterisk*) but not from the SKY hybridisation

Slide pretreatment

Slide pretreatment includes RNase A digestion (Boehringer Mannheim) at 100 μg/ml in 2×SCC for 30 min at 37°C and pepsin digestion (P6887, Sigma) at 0.001–0.003% in 10 mM HCl for 5 min at 37°C. Pepsin incubation times and concentrations vary depending on the age of the metaphase preparations and the amount of cytoplasm and cell debris present near the chromosomes. In general, older specimen require a harsher pretreatment. Too mild a pretreatment will result in low hybridisation signals and high cytoplasmic background, whereas too harsh a pretreatment impairs chromosome morphology and compromises the resolution of hybridisation signals. To maintain chromosome morphology, a postfixation was performed directly after pepsin incubation. The slides are rinsed briefly in PBS and then postfixed in 1% formaldehyde/PBS/50 mM $MgCl_2$ for 10 min. After dehydration through an increasing series of ethanol and air drying, slides are denatured in 70% deionised formamide in 2×SSC at 80°C for 1.5 min, then dehydrated again through an increasing ethanol series and air dried.

Slide pretreatment after Giemsa banding

Metaphase preparations that have been stained for Giemsa banding (G-banding) for classical cytogenetic analysis can be used for SKY analysis. Best results are obtained when G-banding and SKY hybridisation are done on the same day, because Giemsa staining will degrade target DNA over time. G-banded specimens are destained by consecutively dipping in xylene, dipping in methanol and 3-min washes in a decreasing series of ethanol. Postfixation in 1% formaldehyde/PBS/50 mM $MgCl_2$ for 10 min is performed to maintain morphology. Specimens are denatured in 70% formamide/2×SSC on a 80°C slidewarmer for 10–30 s, depending on chromosome morphology, then dehydrated through an increasing ethanol series and air dried.

In situ hybridisation and probe detection

For SKY hybridisation, 15-μl probe cocktails are denatured for 3 min at 80°C and allowed to pre-anneal with human Cot1 for 30 min at 37°C to suppress repetitive DNA sequences. Hybridisation is performed with the15-μl probe mixture over two nights at 37°C in a humidified chamber.

Posthybridisation washes are in 50% formamide/2×SSC at 45°C, 3×10 min, and in 1×SSC at 45°C, 3×5 min. Biotin label is detected using avidin-Cy5 (Amersham) and digoxigenin is detected with mouse anti-digoxin (Sigma) and goat anti-mouse custom-conjugated to Cy5.5 (Amersham). All incubations are for 1 h at 37°C. Immunodetection buffer consists of 4×SSC, 0.1% Tween-20 and 1% bovine serum albumin (BSA; Boehringer Mannheim), pH 7.5. Washes are in the same buffer without 1% BSA, 3×5 min at 45°C. The slides are counterstained with DAPI (80 ng/ml in 2×SSC) for 5 min and covered in *p*-phenylenediamine (Sigma) antifade solution (Platt and Michael 1983).

Microscopy and image acquisition

A Leica DMRBE microscope (Leica, Wetzlar, Germany) is equipped with a SpectraCube SD 200 (Applied Spectral Imaging, Migdal HaEmek, Israel) consisting of an optical head with a special Fourier-transform spectrometer, and a 12-bit cooled CCD camera (Princeton Instruments, Trenton, N.J.) (Garini et al. 1996; Malik et al. 1996). Samples are illuminated with a xenon lamp (Opti-Quip 770/1600, Highland Mills, N.Y.) and imaged with a 100×/N.A. 1.4 or 63×/N.A. 1.25 oil immersion objective (Leica) through a custom-designed triple-bandpass optical filter (Chroma Technology; Brattleboro, Vt.) with broad emission bands (excitation filter 486/28 nm, 565/16 nm, 642/22 nm; emission filter 524/44 nm, 600/38 nm, 720/113 nm; beamsplitter reflection 421–480, 561–572, 631–651; transmission 495–564, 580–620, 660–740). Images are acquired using SKY acquisition software (Applied Spectral Imaging); typically a spectral image is built from 140 frames of 1200 ms. DAPI images are acquired separately.

G-banded samples are imaged with a CCD camera (Photometrics) on the Leica DMRBE microscope set in bright-field mode. To optimise contrast, a neutral density filter and a green filter are inserted in the illumination pathway (Fig. 2A). The X- and Y-stage coordinates of the G-banded metaphases are recorded in order to relocate the same spreads after SKY hybridisation.

Spectral analysis and data presentation

Spectral analysis and classification are performed using SpCube 2.0 visualisation and analysis software (Applied Spectral Imaging). Spectral information for each pixel is retrieved by Fourier transformation of the Sagnac interferogram (Garini et al. 1996; Malik et al. 1996). An emission curve is generated for each pixel of the raw spectral image showing the fluorescence intensities per wavelength. The visualisation software applies an RGB look-up table to the raw spectral image in such a way that emission in the infra-red channel is displayed in red, emission in red is displayed in green, and emission in green is displayed in blue. The display image is based on the fluorescence intensities and unique spectral signature of each chromosome painting probe and, therefore, visualises the ‘real’ SKY hybridisation (Fig. 2B). The display image is used to assess important hybridisation parameters such as signal intensity and homogeneity, and suppression of repetitive DNA regions.

However, analysing the display colours by eye does not allow a definitive colour discrimination. To achieve that goal, a mathematical classification algorithm is applied to discriminate differentially labelled chromosomes unambiguously based on their emission spectrum. In short, pixels are selected from chromosomes that are labelled with only one fluorochrome to define the spectra of the individual fluorochromes for that particular metaphase. The analysis software uses this spectral information to detect the presence or absence of a particular flu-

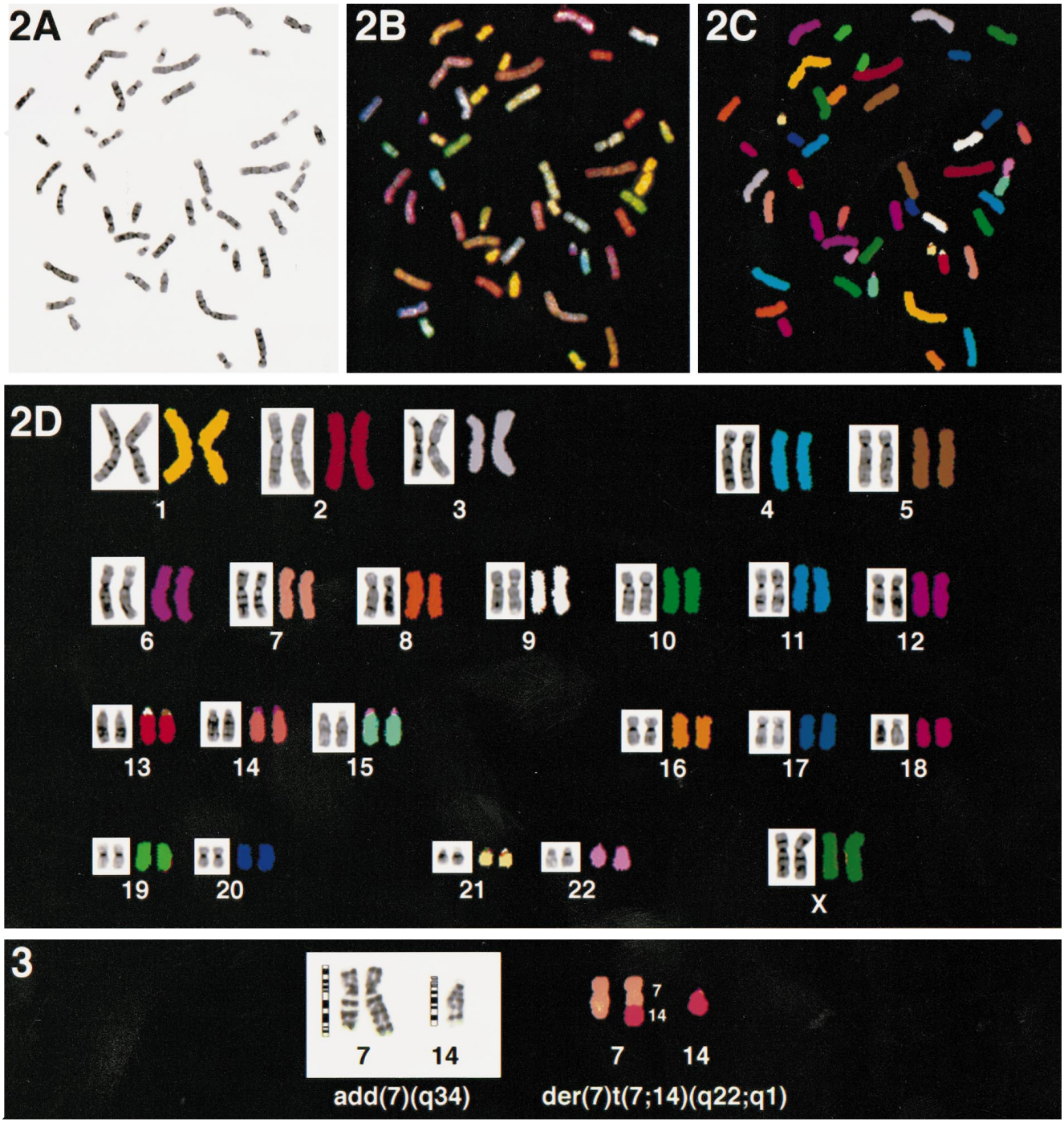

Fig. 2 Metaphases from blood lymphocytes of a karyotypically normal female were G-banded (**A**) and consecutively hybridised for SKY. Chromosomes are shown in SKY display colours (**B**) by setting an RGB look-up table for green, red and infra-red fluorochromes. Pseudo-colours are assigned pixel by pixel by spectral classification (**C**). Pixels similar to the spectrum of chromosome 1 are assigned the colour yellow, chromosome 2 is assigned the colour red, and so on. A karyogram containing the G-banded and classified chromosomes of the same spread is composed electronically (**D**)

Fig. 3 Example of breakpoint refinement in acute myeloid leukaemia. The diagnosis based on G-banding was additional material terminal from 7q34. SKY identified the addition as chromosome 14 and showed that the breakpoint in chromosome 7 was at q22. Courtesy of Veldman et al. (1997)

orochrome in each pixel of the raw spectral image and assigns a pseudo-colour based on the information in the combinatorial labelling scheme. The pseudo-colours, or classification colours, are chosen to maximise contrast (Fig. 2C). An electronically inverted DAPI image, similar to a G-banding pattern, or a G-banding image is aligned with the classified chromosomes (Fig. 2D) to define chromosomal breakpoints.

Image composition and contrast enhancement for this publication were performed using Adobe Photoshop 3.0.

Applications and limitations

SKY will be very useful in clinical cytogenetics for identifying chromosomal rearrangements that cannot be recognised by conventional G-banding, such as translocations that either are subtle or involve regions with similar banding patterns. Using single chromosome paints to identify these markers is time consuming and impractical. To explore the potential of SKY to screen such cases, we have performed SKY without prior knowledge of the chromosomal aberrations on clinical samples from different cytogenetic laboratories that had been previously analysed by G-banding and FISH (Schröck et al. 1996; E. Schröck, manuscript submitted for publication). In all cases, SKY not only confirmed the karyotypes but also revealed unknown abnormalities. For example, SKY detected a balanced translocation t(1;11)(q44;p15.3) in a blood lymphocyte metaphase preparation from a father with a child with mental retardation. G-banding analysis could not detect the fragment of chromosome 11 translocated to chromosome 1 because it was too small. The translocated segments of chromosome 1q and 11p were confirmed by bi-colour FISH using subtelomere-specific probes (National Institutes of Health and Institute of Molecular Medicine Collaboration 1996). The size of the aberration was approximately 1.5 Mb.

In cancer research, SKY will be very helpful for cytogenetic analysis of solid tumours. Tumour cell karyotypes can be very complex. Next to dramatic numerical aberrations, they often contain unidentifiable marker chromosomes of multiple chromosomal origin. As a model study for solid tumour chromosome analysis, we have analysed the breast cancer cell line, SKBR-3 (Schröck et al. 1996) and the cervix carcinoma cell line, SW756 (Garini et al. 1996) by SKY. All marker chromosomes were readily clarified, including a highly rearranged marker chromosome in SKBR-3 that contained an hsr identified as material from chromosome 8, two small insertions from chromosome 3, and the terminal region of chromosome 13 on both ends. Conventional FISH confirmed that the hsr contained multiple copies of the c-myc oncogene, which maps to chromosome band 8q24.

The potential of SKY is further exemplified in haematological malignancies. Fifteen cases of leukaemia and lymphoma were analysed by SKY (Veldman et al. 1997). All cases were previously diagnosed by G-banding and in all instances SKY provided additional information. Figure 3 shows a case of acute myeloid leukaemia (AML) that had been diagnosed by G-banding as a whole chromosome 7 with additional material of unknown origin. SKY not only identified the addition as chromosome 14, but also refined the breakpoint location at 7q22 rather than 7q34. A deletion of 7q22 to q24 has been found in most patients with AML (LeBeau et al. 1996). It is likely that refined karyotype analysis will reveal recurrent chromosomal breakpoints that may become landmarks for a certain tumour or stage of tumour progression.

Over the past few years, mouse models for human tumours have been established to study specific genetic effects in carcinogenesis. Karyotype analysis of these models may contribute important information that could explain the mouse phenotype. However, identifying mouse chromosomes is very difficult because all 20 normal mouse chromosomes are acrocentric and the banding pattern for some of the chromosomes is similar. It goes without saying that analysing abnormal mouse chromosomes requires a special skill which can only be acquired by years of experience. We have developed SKY for mouse chromosomes facilitating karyotype analysis enormously (Liyanage et al. 1996). This will lead to more, and more reliable, cytogenetic data from mouse models.

Besides diagnostic applications in clinical and cancer genetics, SKY has shown its usefulness in defining chromosomal rearrangements that occurred during the course of evolution (comparative cytogenetics; Wienberg et al. 1990). For instance, interspecies SKY hybridisation of human chromosomes to gibbon metaphases showed a distinct pattern of chromosomal rearrangements (Schröck et al. 1996). Based on similarities and differences in such patterns, evolutionary kinship can be determined.

Cytogenetic analysis with SKY requires reference to the nomenclature of chromosome banding, in particular to determine breakpoints. It is therefore essential that banding information is obtained either by electronically inverting a DAPI image or by G-banding. We have shown that sequential G-banding and SKY is possible for lymphocyte metaphases of a healthy donor (Fig. 2). Similarly, tumour metaphases can be analysed (Schröck et al. 1996; M. Macville, unpublished results). The origin of the centromere is important for correct annotation of derivative chromosomes. Due to suppression hybridisation, repetitive sequences, such as all centromere regions, are excluded from SKY analysis. Abnormal chromosomes with unknown centromeric origin, such as whole arm translocations, must therefore be described as a derivative of both chromosomes: der(a;b)(p10;q10). A 24-colour probe cocktail for all human centromeres would be a valuable tool for elucidating centromeric origins. But, more likely, it will find its application in the detection of numerical aberrations in interphase cells, such as amniotic fluid cells in prenatal diagnosis. The reason why this has not been established yet is because some of the currently available centromere-specific probes cross-hybridise to other chromosomes.

The specificity of the whole chromosome painting probes is assured with every new batch of flow-sorted chromosomes in 24 single-chromosome paints. However, with the current probe set, we allow cross-hybridisation of the DNA satellite sequences at the acrocentric chromosomes containing the nucleolus organising regions. As a consequence, these regions do not classify correctly (Fig. 2C) and satellite translocations cannot be identified.

The resolution of SKY is 1–2 Mb, as shown by the t(1;11) described previously. Sometimes, small rear-

ranged chromosomal segments cannot be classified reliably due to strong fluorescent signals of flanking chromosomal regions. In those cases, additional FISH is performed with single painting probes. Intrachromosomal structural aberrations may not always be detected with SKY, because they do not change the colour. Large deletions and amplifications may still be obvious, but small ones may escape the eye all the more when no banding information is available. Inversions can only be picked up by conventional banding methods. Developments are underway to design probe sets that can detect intrachromosomal rearrangements.

Further improvements of the SKY technique will be directed towards improvement of its sensitivity. New fluorochromes and other labelling schemes may be even more suitable for SKY analysis. Reducing the amount of DNA and fluorochromes necessary for one hybridisation will make SKY more cost effective. In that regard, chemical labelling, rather than enzymatic labelling, of large amounts of DNA is an option to explore.

SKY has proven to be a powerful and reliable method for whole genome screening of chromosomal aberrations. It has considerably increased the accuracy of cytogenetic diagnosis in a variety of biological material and is already finding its way into modern cytogenetic laboratories. The robustness of the technique suggests that fully automated karyotyping is within reach.

Acknowledgements The authors wish to thank Y. Garini, I. Bar-Am, D. Wine (Applied Spectal Imaging, Israel) and D. Soenksen (Applied Spectral Imaging, Carlsbad, Calif.) for their continuous support, and S. du Manoir for valuable discussions. This research was supported in part by a Cooperative Research and Development Agreement (NIH-CRADA 9512–8) with Applied Spectral Imaging, Inc.

References

Carter NP (1994) Cytogenetic analysis by chromosome painting. Cytometry 18:2–10

Dauwerse JG, Wiegant J, Raap AK, Breuning MH, Van Ommen GJB (1992) Multiple colors by fluorescence in situ hybridization using ratio-labelled DNA probes create a molecular karyotype. Hum Mol Genet 1:593–598

Florijn RJ, Bonden LAJ, Vrolijk H, Wiegant J, Vaandrager JW, Baas F, Den Dunnen JT, Tanke HJ, Van Ommen GJB, Raap AK (1995) High-resolution DNA fibre-FISH for genomic DNA mapping and colour bar-coding of large genes. Hum Mol Genet 4:831–836

Garini Y, Macville M, Manoir S du, Buckwald RA, Lavi M, Katzir N, Wine D, Bar-Am I, Schröck E, Cabib D, Ried T (1996) Spectral karyotyping. Bioimaging 4:65–72

Hopman AHN, Ramaekers FCS, Raap AK, Beck JLM, Devilee P, Van der Ploeg M, Vooijs GP (1988) In situ hybridization as a tool to study numerical chromosome aberrations in solid bladder tumors. Histochemistry 89:307–316

LeBeau MM (1993) Detecting genetic changes in human tumor cells: have scientists "gone fishing?". Blood 81:1979–1983

LeBeau MM, Espinosa R III, Davis EM, Eisenbart JD, Larson RA, Green ED (1996) Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases. Blood 88:1930–1935

Ledbetter DH (1992) The "colorizing" of cytogenetics: is it ready for prime time? Hum Mol Genet 1:297–299

Liyanage M, Coleman A, Manoir S du, Veldman T, McCormack S, Dickson RB, Barlow C, Wynshaw-Boris A, Janz S, Wienberg J, Ferguson-Smith MA, Schröck E, Ried T (1996) Multicolour spectral karyotyping of mouse chromosomes. Nat Genet 14:312–315

Malik Z, Cabib D, Buckwald RA, Talmi A, Garini Y, Lipson SG (1996) Fourier transform multipixel spectroscopy for quantitative cytology. J Microsc 182:133–140

National Institutes of Health and Institute of Molecular Medicine Collaboration (1996) A complete set of human telomeric probes and their clinical application. Nat Genet 14:86–89

Nederlof PM, Van der Flier S, Wiegant J, Raap AK, Tanke HJ, Ploem JS, Van der Ploeg M (1990) Multiple fluorescence in situ hybridization. Cytometry 11:126–131

Platt JL, Michael AF (1983) Retardation of fading and enhancement of intensity of immunofluorescence by *p*-phenylenediamine. J Histochem Cytochem 13:846–852

Ried T, Landes G, Dackowski W, Klinger K, Ward DC (1992a) Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosome 13, 18, 21, X and Y in uncultured amniotic fluid cells. Hum Mol Genet 1: 307–313

Ried T, Lengauer C, Cremer T, Wiegant J, Raap AK, Van der Ploeg M, Groitl P, Lipp M (1992b) Specific metaphase and interphase detection of the breakpoint region in 8q24 of Burkitt lymphoma cells by triple-color fluorescence in situ hybridization. Genes Chromosom Cancer 4:69–74

Ried T, Baldini A, Rand TC, Ward DC (1992c) Simultaneous visualization of seven different DNA probes by combinatorial fluorescence and digital imaging microscopy. Proc Natl Acad Sci USA 89:1388–1392

Schröck E, Manoir S du, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T (1996) Multicolor spectral karyotyping of human chromosomes. Science 273:494–497

Speicher MR, Ballard SG, Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368–375

Telenius H, Pelmear AH, Tunnacliffe A, Carter NP, Behmel A, Ferguson-Smith MA, Nordenskjold M, Pfragner R, Ponder BAJ (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow sorted chromosomes. Genes Chromosom Cancer 4:267–270

Veldman T, Vignon C, Schröck E, Rowley JD, Ried T (1997) Hidden chromosome abnormalities in haematological malignacies detected by multicolour spectral karyotyping. Nat Genet 15: 406–410

Wiegant J, Wiesmeijer CC, Hoovers JMN, Schuuring E, d'Azzo A, Vrolijk J, Tanke HJ, Raap AK (1993) Multiple and sensitive fluorescence in situ hybridization with rhodamine-, fluorescein-, coumarin labeled DNAs. Cytogenet Cell Genet 62: 73–76

Wienberg J, Jauch A, Stanyon R, Cremer T (1990) Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics 8:347–350

Zimonjic DB, Popescu NC (1994) An improved procedure for chromosome preparations from solid tumors. Cancer Genet Cytogenet 72:161